Disrupting SIV Reservoir Seeding by Targeting Stemness Pathways in Rhesus Macaques

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BACKGROUND

Latently HIV-infected CD4⁺ T cells persist through proliferation. We previously showed that inhibition of proliferation and induction of differentiation of the long-lived, self-renewing central memory (CM) and stem cell memory (SCM) CD4⁺ T cells can be achieved in SIV-infected rhesus macaques (RMs) treated with antiretroviral therapy (ART) through modulation of the Wnt pathway [1].

Here, we evaluated a combined the approach targeting the Wnt and Notch pathways during acute SIV infection of RMs to disrupt viral reservoir establishment.

STUDY DESIGN

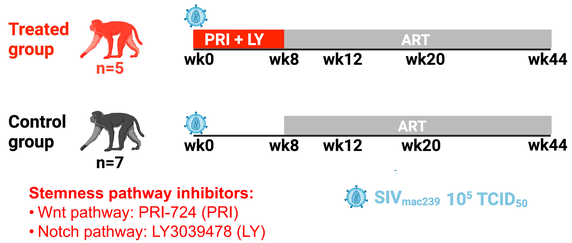


Fig. 1. Experimental study design. Five RMs were infected intravenously with 10^5 TCID50 (50% tissue culture infective dose) of SIV_{mac239} before receiving the combined treatment PRI-724 (20 mg/kg, subcutaneously, daily) + LY3039478 (2.5 mg/kg, orally, three times per week) for 8 weeks. ART was initiated 8 weeks post infection and maintained for the duration of the study. A historical group of 7 RMs that had followed the same experimental design was used for comparison.

METHODS

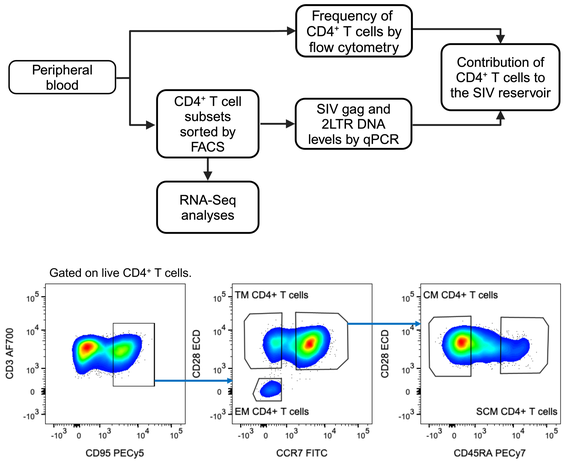


Fig. 2. Gating strategy for cell sorting of CD4⁺ T cell subsets. Representative flow cytometry dot plots showing the gating strategy used for cell sorting of Stem Cell Memory (SCM: CD95+ CCR7+ CD45RA+), Central Memory (CM: CD95+ CCR7+ CD45RA-), Transitional Memory (TM: CD95+ CCR7- CD28+) and Effector Memory (EM: CD95+ CCR7- CD28-) CD4⁺ T cells from peripheral blood.

Pharmacological modulation of signaling pathways regulating T cell proliferation during acute SIV infection alters SIV reservoir distribution with a targeted effect on central memory CD4⁺ T cells.

Transient reduction of the contribution of the CM subset to the CD4⁺ T cell pool and SIV reservoir in the PRI-724 + LY3039478 treated group

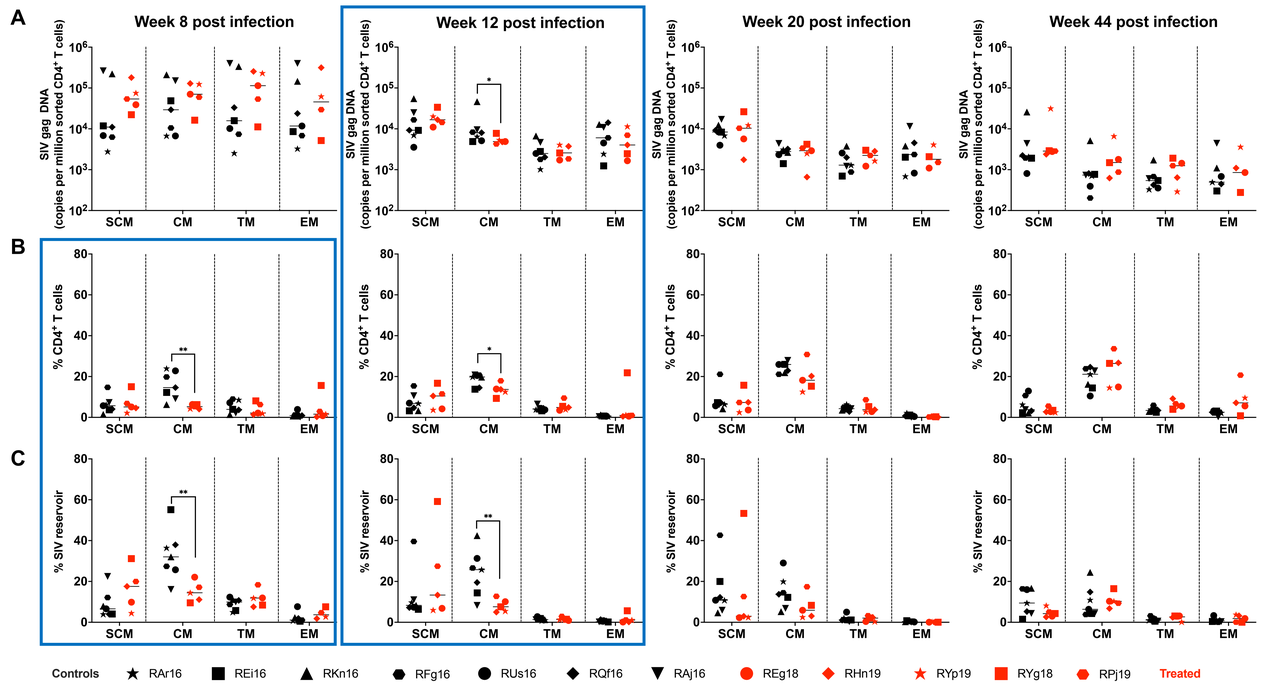


Fig. 3. Impact of PRI-724 + LY3039478 combined treatment on SIV reservoir distribution. Longitudinal assessment of (A) SIV gag DNA levels in the subsets of CD4⁺ T cells, (B) the relative contribution of CD4⁺ T cell subsets to the total peripheral CD4⁺ T cell pool and (C) the relative contribution of CD4⁺ T cell subsets to SIV reservoir at weeks 8, 12, 20 and 44 post infection. Horizontal bars represent the median. A two-sided Mann Whitney U-test was used to compare values between groups. *P < 0.05, **P < 0.01.

Higher SIV gag/2LTR ratio in memory CD4⁺ T cell subsets in the PRI-724 + LY3039478 treated group

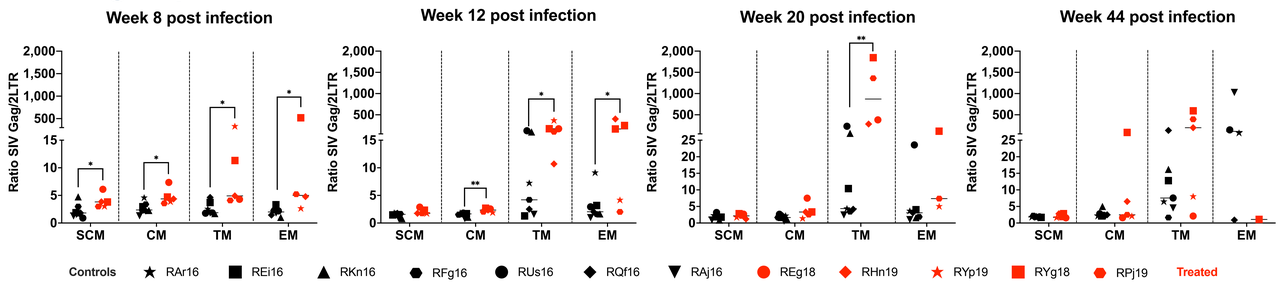


Fig. 4. Impact of PRI-724 + LY3039478 combined treatment on SIV Gag/2LTR ratio. Longitudinal assessment of the SIV Gag/2LTR (long terminal repeat) ratio in peripheral CD4⁺ T cell subsets at weeks 8, 12, 20 and 44 post infection. Horizontal bars represent the median. A two-sided Mann Whitney U-test was used to compare values between groups. *P < 0.05, **P < 0.01.

ACKNOWLEDGEMENTS

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1. Mavigner, M., et al., Pharmacological Modulation of the Wnt/beta-Catenin Pathway Inhibits Proliferation and Promotes Differentiation of Long-Lived Memory CD4⁺ T Cells in Antiretroviral Therapy-Suppressed Simian Immunodeficiency Virus-Infected Macaques. J Virol, 2019.

Changes in gene expression in CM CD4⁺ T cells from the PRI-724 + LY3039478 treated group

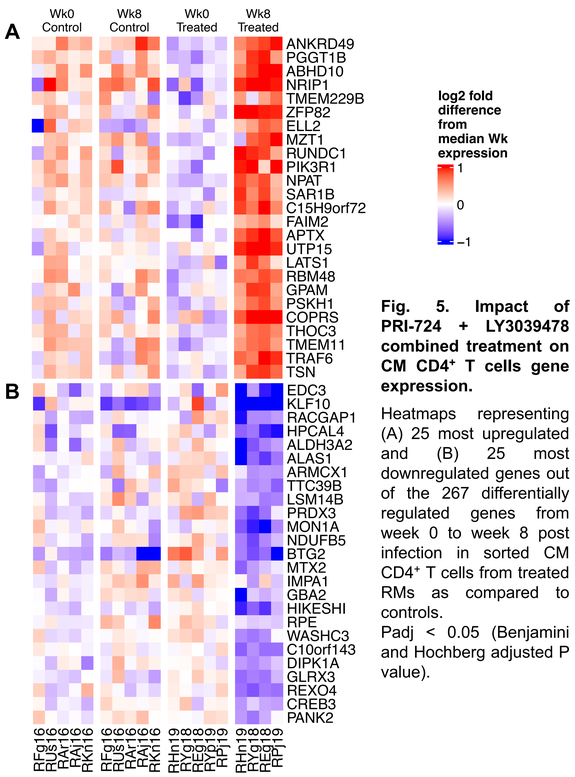


Fig. 5. Impact of PRI-724 + LY3039478 combined treatment on CM CD4⁺ T cells gene expression.

Heatmaps representing (A) 25 most upregulated and (B) 25 most downregulated genes out of the 267 differentially regulated genes from week 0 to week 8 post infection in sorted CM CD4⁺ T cells from treated RMs as compared to controls.
Padj < 0.05 (Benjamini and Hochberg adjusted P value).

CONCLUSIONS

- Transcriptomic analyses showed that PRI-724 + LY3039478 treatment altered the gene expression profile of CM CD4⁺ T cells, including genes associated with Notch and Wnt pathways and with cell proliferation and differentiation.
- A higher SIV gag/2LTR ratio was observed in all memory CD4⁺ T cell subsets in treated RMs as compared to controls, suggesting an impact of the experimental treatment on reservoir cell proliferation/differentiation.
- The combined inhibition of the Wnt and Notch pathways transiently altered SIV reservoir distribution with a decreased contribution of long-lived CM CD4⁺ T cells.
- Combination treatments targeting pathways regulating T cell proliferation represent a novel strategy to alter SIV/HIV persistence.